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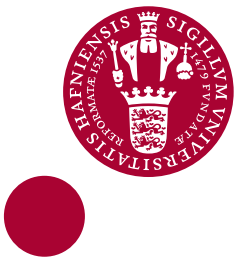

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Published in:
Journal of Applied Phycology

DOI:
10.1007/s10811-018-1632-x

Publication date:
2019

Document version
Peer reviewed version

Citation for published version (APA):
Mouritsen, O. G., Duelund, L., Petersen, M. A., Hartmann, A. L., & Frøst, M. B. (2019). Umami taste, free amino acid composition, and volatile compounds of brown seaweeds: [Incl. Correction]. *Journal of Applied Phycology*, *31*(2), 1213-1232. https://doi.org/10.1007/s10811-018-1632-x

Download date: 04. May. 2023

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Umami taste, free amino acid composition, and volatile compounds of brown seaweeds

Ole G. Mouritsen*[1], Lars Duelund[2], Mikael Agerlin Petersen[3], Anna Loraine Hartmann[1], Michael Bom Frøst[1]

Abstract

Umami taste is associated with deliciousness and was first suggested as a basic taste by Kikunae Ikeda in 1909 when he discovered that the brown seaweed *konbu* (*Saccharina japonica*), used to provide aqueous extracts for the Japanese soup stock *dashi*, contains very large amounts of free glutamate. We have performed a comparative analysis of the free amino contents of 20 different species of brown seaweeds used for human consumption from around the world, belonging to the 12 genera *Nereocystis, Macrocystis, Laminaria, Saccharina, Undaria, Alaria, Postelsia, Himanthalia, Ecklonia* (former *Eisenia*)*, Sargassum, Fucus,* and *Corda.* We furthermore measured mineral and iodine contents as well as identified a range of volatile compounds and estimated their influence on the perception of umami taste. The results provide a basis on which chefs and food producers can control umami sensation in food items using some of the most popular species of edible brown seaweeds.

Keywords: Umami; brown seaweeds, taste, HPLC-MS, GC

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1. Introduction

Whereas a great variety of different seaweeds, in particular brown seaweeds, for millennia have been used as a staple food source in Asia (Arasaki, 1983) the tradition for using seaweeds as part of the daily fare in Europe and the Americas is maintained in only a few places (Mouritsen, 2013; Bird, 2015; O'Connor, 2017; Mouritsen et al., 2018). However, due to increasing awareness of sustainable marine food resources, a healthy and nutritious diet, and not least a growing interest in the gastronomical value of algae among both chefs and food companies it appears that consumers in the Western world are becoming more tuned to eating seaweeds and products derived from seaweeds. A key issue in this development is a movement beyond a focus on nutrition and health towards an

appreciation of the taste and flavour of foodstuff based on seaweeds (Mouritsen, 2017; Pérez-Lloréns et al., 2018).

In Southeast Asia seaweeds are recognized for their particular texture or mouthfeel and their capacity for eliciting umami taste. In the Western world the connotation to seaweeds is often one related to the off-putting odours of decaying seaweeds washed ashore. Also, some consumers find that particularly brown seaweeds from the Fucales and Laminariales orders have too much and overpowering marine flavours. Interestingly, the essence of deliciousness in the sensory perception of food is often associated with the umami taste of the food in question. Umami is a basic taste (along with sweet, sour, salty, and bitter) which was proposed in 1909 by Kikunae Ikeda (Ikeda, 2002) when he discovered that the large brown seaweed *konbu* (*Saccharina japonica*) contains very large amounts of free glutamate to which Ikeda attributed a new, fifth basic taste, umami. In the Japanese cuisine, as well as in other South Asian food cultures, *konbu* is used to produce an aqueous extract that is the basis of a soup stock, *dashi*, whose amino acid composition is rather singularly dominated by free glutamate and free aspartate, both of which are now known to bind to and stimulate the umami receptors (Zhang et al., 2008; Mouritsen and Khandelia, 2012). A traditional full Japanese *dashi* contains in the addition to an extract of *konbu* also an aqueous extract of a special fish product, *katsuobushi*. *Konbu* together with *katsuobushi* provide a synergy in the umami sensation by the simultaneous presence of free glutamate and free inosinate (Mouritsen and Styrbæk, 2014). A *dashi* only based on *konbu* is called *konbu-dashi.* The present paper focusses on such *dashis* based solely on seaweed extracts.

In the Japanese cuisine, umami taste from *konbu* and *dashi* is not only used for soups but also to flavour other dishes, in particular vegetables (Fuji, 2005; Antony et al., 2014; Japanese Culinary Academy, 2016), rendering it possible to produce delicious dishes and meals with less sugar, less salt, and less fats (Mouritsen and Styrbæk, 2014). However, deliciousness is not only a matter of umami but equally well lucky combinations of both food taste and aromas, as well an appealing mouthfeel (Mouritsen and Styrbæk, 2017). Moreover, the use of a particular seaweed in a certain dish need not correlate in a simple manner with the sensation of umami. First of all, the actual contents of umami compounds vary from one species to another, and also often between subspecies of, e.g., *Saccharina japonica* (Ninomiya, 1998; Blumenthal et al., 2009; Mouritsen et al., 2012). Secondly,

84 the experienced sensation of umami on the palate depends on the presence of other taste and aroma
85 compounds (Maga, 1983; Fuke, 1996), in particular certain free nucleotides such as inosinate.

86

87 In order to evaluate the umami potential of seaweeds for use in foodstuff it is therefore important to
88 have information about the actual chemical composition of the seaweeds as well data from a sensory
89 analysis. It turns out that surprisingly little information is available in the scientific literature on the
90 amino acid composition of seaweeds (Sánchez-Machado et al., 2004; Dawczynski et al., 2006;
91 MacArtain et al., 2007; Kurihara, 2009; Pereira, 2011; 2016; Pomin, 2012; Mouritsen et al., 2013;
92 Mouritsen, 2013) and even less when combined with quantitative sensory studies. Despite this lack
93 of information it is often stated quite generally among many chefs that seaweeds elicit umami taste.
94 The purpose of the present paper is to derive quantitative data for the chemical composition of a series
95 of brown seaweeds that are often used for foodstuff around the world and combining these data with
96 a sensory analysis, thereby unravelling the actual umami potential of the seaweeds. In addition, we
97 identify and quantitate volatile aroma compounds in some of the seaweeds and relate the intensity of
98 the perception of umami to the simultaneous presence of aroma compounds. With regard to the
99 umami potential of red seaweeds and a comparison with a few selected brown species, an earlier
100 study (Mouritsen et al., 2012; 2013) has investigated dulse (*Palmaria palmata*) and found that this
101 species has rather high levels of free glutamate that contribute to its sensorily perceived umami
102 potential.

103

104 We have selected a series of 20 different species of brown seaweeds used for human consumption
105 from around the word, belonging to the 12 genera *Nereocystis, Macrocystis, Laminaria, Saccharina,*
106 *Undaria, Alaria, Postelsia, Himanthalia, Ecklonia* (former *Eisenia*)*, Sargassum, Fucus,* and *Corda.*
107 For some of the species we have used material sourced from different waters, making our comparative
108 analysis containing 36 samples of dry seaweed. We included a 37th sample in the analysis being a
109 special salt precipitate of bull kelp (*Nereocystis leutkeana*) produced during the drying process of the
110 freshly harvested seaweed. This special product has been included in order to evaluate its gastronomic
111 value and as a possible source of saltiness and umami taste.

112 Our study is aimed at investigating the umami potential of aqueous extracts (in the following just
113 termed *dashi*) of the different seaweeds since we focus on the use of these seaweeds as seasoning

agents in e.g., soups, marinating media, dressings, sauces, and simmering liquids. We have measured the complete amino acid profile as well as the contents of sodium, potassium, and of iodine. Based on the compositional profile, 16 samples were specifically selected and investigated in order to identify characteristic volatile compounds. The same 16 samples were subsequently subjected to a sensory analysis from which we have determined the influence of free amino acids, mineral content, iodine, as well as various aroma compounds on the perceived sensation of umami in aqueous extracts of the seaweeds.

2. Materials and Methods

2.1 Seaweeds and extraction methods

The various seaweed materials investigated have been either purchased commercially or obtained directly from harvesters. The 37 different seaweed samples are listed in Table 1, along with an indication of their origin.

The taste and aroma compounds were extracted in tap water according to a previously described procedure (Mouritsen et al., 2012). Ordinary, non-filtered tap water (Odense, Denmark; water hardness = 15°dH) was used. All extractions were based on 5 g of dry seaweed in 250 ml of water placed in a plastic bag sealed under vacuum pressure (sous-vide) and immersed over a period of 45 minutes in a water bath at 60ºC. All extractions were performed in duplicate by taking two independent samples from the same batch of material. The seaweed salt precipitate, which is a white crystalline powder with a few pieces of dried bull kelp, is subjected to the same extraction procedure as the dry seaweed samples.

It has earlier been shown (Mouritsen et al., 2012) for dulse (*Palmaria palmata*), sugar kelp (*Saccharina latissima*), and *konbu* (*Saccharina japonica*) that the use of well-controlled extraction techniques as applied here, in contrast to traditional recipes using open pans and near-water boiling conditions, improves the extraction efficiency for free amino acids from seaweeds without compromising the flavour. Whereas the extraction temperature has a definite influence on the overall taste of the extract, in particular the bitter notes developed at the higher temperatures, the amounts of free amino acids appear to be little sensitive to whether the extraction temperature is 60°C or 100°C.

Similarly, the earlier work (Mouritsen et al., 2012) did not detect any significant dependence of water hardness on the amount of released umami-flavouring free amino acids. The extracts were cooled and frozen immediately after extraction and only thawed just before analysis and tasting.

For convenience, the contents of free amino acids, sodium, potassium, and iodine are given in unit per 100 mL extract since the use of the data will predominantly be in the context of evaluating the contents in liquid solutions (*dashi*). Assuming that the seaweeds swell approximately a factor of eight in water and that their density after complete swelling is approximately the same as that for water, the data given for contents per 100 mL extract can be converted to contents per gram dry material by multiplying by a factor 255/[(255-40)×5]=0.24.

2.2 Chemicals

Acetonitrile was from VWR (Ratnor, PA). Formic acid and NH_4HCO_2 were from Sigma Aldrich (St. Luis, MO). Amino acid and aroma standards were from Sigma-Aldrich. Trace-metal grade HNO_3 was from Normatom, VWR. Trace-metal standard was from Traceselect, Sigma Aldrich.

2.3 Amino acid analysis

Detection and quantification of free amino acids was performed by HPLC-MS as described in Mouritsen et al. (2017) using a Shimadzu LCMS 2020 MS equipped with an electrospray interface (ESI). The HPLC consisted of a DGU20-A5 on-line degasser, two LC-20 AD pumps, a high-pressure mixer, an SIL20A- HT autosampler, a CTO 10 Column oven, and an SPD-20A UV detector, all from Shimadzu (Holm & Halby Brøndby, Denmark). The separation of the 17 different free amino acids was performed on a 75x3mm Imtak Intrada amino acid column (Imtakt Corporation, Kyoto, Japan) by a gradient of acetonitrile with 0.1% formic acid and 100 mM NH_4HCO_2 over a time course of 30 min. All analyses were carried out in duplicate.

The individual amino acids were detected by selected ion monitoring (SIM) at appropriate *m/Z* values and were quantified by comparison with a standard curve prepared from a commercially available

amino acid standard. All samples were diluted ten times with 0.1 M HCl and filtered through a 0.2 µm RC syringe filter before analysis. Due to their similar mass, Ile and Leu are difficult to separate in some samples and we have therefore for convenience only measured their sum. Cystine is measured as a dimer Cys_2 but all data reported labelled by Cys_2 have been renormalized to monomer concentration. All analyses were carried out in duplicate.

2.4 Volatile compound analysis

Analyses were carried out in duplicate. Twenty mL of sample was equilibrated to 37°C in gas washing flasks in a circulating water bath and then purged with nitrogen (150 mL min^{-1}) for 60 min with magnetic stirring (200 rpm). Volatile compounds were collected on Tenax-TA traps. The traps contained 200 mg of Tenax-TA with mesh size 60/80 (Markes International, Llantrisant, UK). After purging, water was removed from the traps with a flow of dry nitrogen (150 mL min^{-1} for 20 min).

The trapped volatiles were desorbed using an automatic thermal desorption unit (TurboMatrix 350, Perkin Elmer, Shelton, USA). Primary desorption was carried out by heating the trap to 250°C with a flow (50 mL min^{-1}) of carrier gas for 15.0 min. The stripped volatiles were trapped in a Tenax TA cold trap (30 mg held at 5°C), which was subsequently heated at 300°C for 4 min (secondary desorption, outlet split 1:10). This allowed for rapid transfer of volatiles to a gas chromatograph-mass spectrometer (GC-MS, 7890A GC-system interfaced with a 5975C VL MSD with Triple-Axis detector from Agilent Technologies, Palo Alto, California) through a heated (225°C) transfer line.

Separation of volatiles was carried out on a ZB-Wax capillary column 30 m long x 0.25 mm internal diameter, 0.50 µm film thickness. The column pressure was held constant at 2.3 psi resulting in an initial flow rate of 1.4 mL min^{-1} using hydrogen as carrier gas. The column temperature programme was: 10 min at 30°C, from 30°C to 240°C at 8°C min^{-1}, and finally 5 min at 240°C. The mass spectrometer was operating in the electron ionisation mode at 70 eV. Mass-to-charge ratios between 15 and 300 were scanned. Peak areas and mass spectra were extracted from the chromatograms using the PARAFAC2 based software PARADISe (University of Copenhagen, Copenhagen, Denmark) (Johnsen et al., 2017) and mass spectra were identified using the NIST11 database. Peak areas were used as relative measures of concentration. Volatile compound identification was confirmed by

comparison with retention indices (RI) of authentic reference compounds or retention indices reported in the literature.

2.5 Sodium, potassium, and iodine analysis

Sodium and potassium contents were determined by flame atomic-absorbance spectroscopy (AAS) on a Shimadzu AA7000 spectrometer equipped with an ASC-7000 auto-sampler (Holm & Halby Brøndby, Denmark) as described in Mouritsen et al. (2017). Samples were diluted as needed with trace-metal grade 1 M HNO_3 before analysis. The concentrations were calculated from a standard curve obtained from the analysis of a commercial standard. All analyses were carried out in duplicate.

Iodine was determined by a the Sandell-Kolhoff reaction in a 96 well plate version as described by Strydom and Jooste (2011). All measurements where done in a BMG Labtech Fluostar Omega plate reader (BMG Labtech GMBH, Ortenberg, Germany). Analyses were carried out in duplicate.

2.6 Sensory evaluation

Initially, potential panellists were screened for their sensitivity to monosodium glutamate (ISO, 2011). The purpose was to ascertain that they could perceive the compound and understand the sensory descriptor. The sensory training consisted of two stages. The first stage was a descriptor-development session with a duration of approximately 2 hours. In the descriptor-development session, the panellists came up with all the descriptors they could think of to describe the samples. The panel leader chose the descriptors to use for the training based on consensus among the panellists. In the second stage (2 sessions of 1½-2 hours) the panellists were trained for the assessment of *dashi*. In each session a subset of the samples presented in the evaluations were used. 23 descriptors were chosen for the final evaluation (Table 4). These included descriptors for appearance, aroma, flavour, mouthfeel, and taste.

The training was realized with panellists seated at individual tables and not disturbing each other during tastings. The sensory evaluations were performed in a sensory test facility, with panellists seated at individual booths and not disturbing each other during tastings. The descriptive sensory

231 analysis was conducted with 8 paid external panellists in 6 sessions. The analysis was carried out in
232 two different conditions – with and without olfactory input, three sessions of each variant. In each
233 session, 16 samples were served (i.e., three sensory replicates under each of the two conditions). Prior
234 to serving, the samples were heated for 15 minutes in a steam oven (Rational, Bent Brandt, Denmark)
235 and kept at a serving temperature of 60°C in a thermostat (Termaks KB8182, Nino Lab, Denmark).
236 The purpose of intentionally omitting olfactory input in half of the sensory tests was to evaluate its
237 effect on central sensory properties such as umami. This part of the analysis is treated in a separate
238 article focusing on multi-modal interactions (Frøst et al., in preparation).

239

240 *2.8 Data analysis*

241 Data were scrutinized by analysis of variance (ANOVA) and multivariate data analysis (ANOVA–
242 partial least squares regression (A–PLSR), and PLSR). For chemical analysis, fixed factor analysis
243 was performed with samples as fixed factors. For data obtained by HPLC, n=37, and for GC, n=16.
244 The samples subjected to GC and sensory analysis were the same. For sensory analysis, mixed model
245 ANOVA for individual descriptors was performed with samples (n=16) as fixed factors and panellists
246 (n=8) as random factors. This method is commonly applied to data from descriptive analysis (Lawless
247 & Heymann, 2010; Næs & Langsrud, 1998). Least significant differences at 5% level (LSD 5%) were
248 estimated based on Mean Square Error. Mean ratings over panellists from each replicate was used for
249 A–PLSR.

250

251 A–PLSR is a multivariate regression method where the effect of design factors on the response
252 variables (here, the physico-chemical and sensory descriptors, respectively) is evaluated (Martens
253 and Næs, 1989; Martens and Martens, 2001). The method avoids multi-collinearity problems by
254 modelling latent variables representing the main variance common for the variables. The method
255 evaluates effects of the experimental design variables on physico-chemical characterisations and
256 sensory properties. Here it is used as a graphical alternative to one-way ANOVA. Mean ratings over
257 panellists from each replicate were used for A–PLSR. For all physico-chemical analysis, individual
258 data from each of the 3 or 4 replicates was used. For multivariate analyses, cross-validation was
259 performed, leaving out one replicate at a time (Martens and Næs, 1989). Jack-knifing with replicates
260 served as the validation tool for all A-PLSR, comparing the perturbed model parameter estimates

261 from cross-validation with the estimates for the full model (Martens and Martens, 2000). For sensory
262 data, mean over panellists in each replicate was used for cross validation (see e.g. Johansen et al.
263 (2008) for advantages of this approach).

264

265 The relationship between physico-chemical characteristics and sensory properties of the *dashis* is
266 analysed by PLSR regression carried out on mean data. For physico-chemical measurements mean
267 data are means over replicates of measurements (3 or 4) replicates unless otherwise stated). For
268 sensory data mean data are averaged over panellists (n=8) and replicates (n=3).

269

270 **3. Results and discussion**

271 Some caution should be exercised when comparing data for both different and the same seaweed
272 species from different sources, because the sample material can vary depending on the growing
273 conditions and the time of harvest. Furthermore, in the case of the amino acid analysis the methods
274 reported in the literature can differ as well. The latter can be particularly troublesome for analysis of
275 extracts of glutamate from brown seaweeds, because it is known that their alginate and salt contents
276 can interfere with the derivation of the amino acids when using classic HPLC methods (Bergeron &
277 Jolivet, 1991). Moreover, different workers have used different amounts of seaweed for their
278 extractions, and the extraction efficiency may depend on the relative amount of water used. In the
279 present work, we used about twice the amount of dry seaweed per litre of water compared with many
280 classic Japanese *dashi* recipes.

281

282 From the data in Table 2 it is found that the contents of all amino acids except cysteine varied
283 significantly between the 37 samples. The contents of iodine, sodium, and potassium also vary
284 substantially across the samples. Scrutiny of the table and the confidence intervals shows that all
285 samples are significantly different from each other.

286

287 In order to further investigate in which sense the different samples are significantly different we
288 present in Fig. 1 correlation loading plots of dimensions 1 and 2 (Fig. 1a) and dimensions 3 and 4
289 (Fig. 1b) for all 37 samples using the concentrations in Table 2 as variables. The plot gives an

overview of the interrelationship between samples and variables. From Fig. 1a it can be seen that two samples are located away from the remaining samples along dimension 1, 01Nl (bull kelp) and 14Up (*wakame* of Japanese origin). These are the samples with the highest content of all of the amino acids found in the right part of Fig. 1a. The samples in the upper part of Fig. 1a are high in glutamate and iodine and are mainly Japanese *konbu* (*Saccharina japonica*) samples. Samples toward the lower left part of the figure have the lowest content of all the compounds, except sodium. From Fig. 1b it can be seen that dimension 3 and 4 combined separates samples that are high in sodium, exemplified by 16Ae and 30Eb) from those high in iodine (exemplified by 05Lg). The optimal number of dimensions is 4, and the explained variation for the four dimensions is about 45%. This implies that a large part of the differences between samples is not accounted for. The grouping of the samples does not show a systematic pattern based on origin. But there is a grouping based on species, particularly for the different samples of *konbu*.

Table 3 lists the mean values for 49 aroma compounds identified for the 16 samples that were subjected to GC analysis. A larger number of compounds were identified, but all data analysis only includes these 49 variables, as they are the compounds that exhibited significant differences between the 16 samples. Among other compounds found in significant amounts, but not dealt with in the comparative analysis, are typical degradation products of fatty acids, such as propanal, nonanal, decanal, and undecanal. We have also detected acetone, ethyl acetate, ethyl propanoate, methyl methacrylate, 3-heptanon, butanol, butyrolacton, and acetophenone. All these compounds have fairly low detection threshold values and are furthermore well-known aroma compounds found in many kinds of foodstuff. Scrutiny of Table 3 shows that judged from the composition of the 49 selected aroma compounds all samples are significantly different from each other. There are few previously published records of volatile components from brown seaweeds (Boonprap et al., 2006; Keng et al., 2013) and none for *dashi*, so the magnitude of the intra- and interspecies variation is difficult to assess.

The results of the A-PLSR show that 4 dimensions are the optimal solution to extract all of the systematic variance in the data set. The 4 dimensions explain 73% of the variance in the data. Figs. 2a and b show correlation loading plots from the A-PLSR for the 4 significant dimensions. The multivariate analysis demonstrates that one sample has a distinctly different aroma compound profile from the rest. It is 15Up, a sample of *wakame* harvested in Brittany in France. The sample is located

in the far left part of the figure. It has an exceptional profile of volatiles with a very high concentration of many compounds that are very low in all other samples. The remaining 15 samples are distributed along dimension 2, but not in a systematic manner. Dimensions 3 and 4 are shown in Fig. 2b which differentiates sample 29Pp (sea palm from California) from a centre group and the aroma compounds that characterize them, namely 1-octanol, octanal, and 1-heptanol. In the top part of the figure, samples 01Nl and 03Ls are located, which are respectively bull kelp and North Pacific *konbu* (*Laminaria setchelli*), both from Vancouver Island. They have a higher concentration of 2,3-butanedione and 3-methylbutanal. In the lower part of dimension 4 samples 30Ebi (*arame* from Japan) and 32Fv (bladderwrack from Denmark) are located. They are mostly characterized by lower levels of the compounds in the upper part. Scrutiny of differences among samples of same species is only relevant for *konbu* (*Saccharina japonica*, abbreviated Sj in all figures) harvested from different locations. Although the 5 different samples are located closely together in Fig. 2a, scrutiny of Table 3 reveals that significant differences exist among those samples. The differences are most pronounced in compounds such as (Z)-2-nonen-1-ol; (E)-2-nonenal; 1-octen-3-one; 6-methyl-5-hepten-2-one; (Z)-6-octen-2-one. Thus, it is clear that differences exist between harvest locations of *konbu*. However, the intra-species differences in *konbu* are much smaller than the between different species.

3.1 Free amino acids

Table 2 lists the mean values from the analysis by HPLC of the amino acid content. The two most significant observations pertain to the contents of Ala and Glu that for most species are significantly higher than the contents of the remaining free amino acids. Ala has a sweet taste and Glu elicits umami taste by stimulating the umami receptor. Asp also has umami taste but the potency is much less than that of Glu (Li et al., 2002; Chandrashekar et al., 2006). In any case, the content of Asp is rather low in most species included in the present study.

In Fig. xxx the contents of Glu are listed for all samples and arranged according to magnitude. This figure clearly exposes the different types of *konbu* as high in Glu. The umami potential of the different seaweeds based solely on the glutamate content can easily be judged from this figure. Similarly, Fig. 4 displays the contents of Asp with the different samples arranged in the same order as in Fig. 4. It appears from this figure, that the various samples of *konbu* are generally significantly lower in

aspartate contents than most of the other species. The dominance of Glu over Asp is very pronounced in *konbu*.

Glu is particularly high in the range of different types of *konbu* (*Japonica saccharina*) whereas Ala is high in bull kelp, *wakame*, macrokelp, the *Laminaria*-species, as well as sea palm (cf. Fig. 4). Some samples of sugar kelp also have substantial contributions of Ala. It is of particular interest to compare the different types of *konbu* with respect to their content of Glu. It is well known that different subspecies of Japanese *konbu* grown at various locations around the coast of Hokkaido have very different capacity to impart umami taste to *dashi* (Japanese Culinary Academy, 2016), a fact that is often reflected in their market price. In accordance with previous studies we found that the level of free Glu is highest in *Ma-konbu* and lowest in *Hidaka-konbu*. *Rishiri-konbu* and *Raushu-konbu* are in between, but there are some minor differences for different harvesting sites and vendors. It is interesting to note, that the Glu contents of the related species sugar kelp (*Saccharina latissima*) is much less than in Japanese *konbu*, a finding that has been reported before (Mouritsen et al., 2012). Also the various *Laminaria*-species have low Glu contents. The sample of Japanese *wakame* has a noticeable low level of Glu and conversely a high level of Ala. It is interesting in this context to mention the Glu content in a *dashi* prepared from the red seaweed dulse (*Palmaria palmata*) using the same extraction techniques as in the present paper. It was found that the dulse *dashi* has a rich umami taste and contained typically 10-40 mg Glu/ 100 mL depending on the site of harvest (Mouritsen et al., 2012).

3.2 Volatile compounds

Table 2 and Table 3 lists the mean values for analysis by HPLC for 37 samples and GC of volatile components for 16 samples, respectively. An additional A-PLSR (Fig. 3) was performed to investigate if there are different patterns for sample differences between the two types of chemical analysis for those samples. Fig. 3 reveals that similar to the GC-analysis, the French *wakame* (15Up) is quite different from the remaining 15 samples. However, there are major differences in the pattern compared to the GC analysis (Fig. 2a). The Vancouver Island Bull kelp (01Nl) can be singled out as the sample most different from all other, measured by HPLC, whereas it is centred among all samples

when analysed by GC. Hence, already from this analysis we can concur that there are major differences in the characterization of samples using HPLC and GC.

3.3 Sodium, potassium, and iodine

Table 2 lists the mean values from the AAS-analysis for the contents of sodium and potassium in the different samples. The data reflects a general finding with most seaweeds (Mouritsen, 2013) that potassium salts outbalance sodium salts, often with a factor 2-3 in concentration. Species with very high potassium contents include bull kelp, macrokelp, tangleweed, oarweed, thongweed, *arame*, and some samples of sugar kelp as well as *konbu*. The set of data in Table 2 has an outlier with very low levels of potassium, 15Up, which is a *wakame* sample from Brittany. We have no explanation for this anomalies and since the extractions and HPLC measurements have been done repeatedly on different samples of the same batch it is conceivable that the low values are correct.

The analysis for iodine only accounts for the sum of the water-soluble, inorganic forms such as iodide and iodate. Any organically bound iodine is expected to remain in the seaweed tissue. The contents of iodine in the different samples are as shown in Table 2 varying by more than a factor of 300 going from very low contents (41 µg/100 mL) in some *wakame* samples to very high (2.000-13.000 µg/100 mL) for samples in the Laminariales order (the so-called kelps, such as *konbu*, sugar kelp, tangleweed, and oarweed). Macrokelp (02Mp) is in the same tier as the Laminariales with regard to iodine content.

The data for the iodine contents is displayed in Fig. xxx where the samples are ordered with respect to iodine concentration. The *dashis* from winged kelp, *arame*, *hijiki*, thongweed, and *wakame* are fairly low in iodine, whereas for bull kelp, sea lace, sea palm, and the various wracks (Fucales) they are a somewhat higher. As mentioned above, the kelps are very high in iodine. The data reported here is generally in accordance with findings in the literature (Arasaki & Arazaki, 1983; Mabeau & Fleurence, 1993; Rupérez, 2002; Teas et al., 2004; Holdt & Kraan, 2011), although there are variations as expected, considering the fact that the levels of accumulated iodine is dependent on growing conditions.

409 Iodine is an essential element for the synthesis of the thyroid hormones triiodothyronine and thyroxine
410 (Braverman, 1994) and this is the only known functional purpose iodine serves in the humane body.
411 The daily requirement is about 2 µg per kilo of body weight, i.e. about 140 µg for a normal adult. A
412 diet deficient in iodine can lead to adverse hormonal, hypothyroidal effects such as goiter and
413 cretinism, and in rare cases excessive amounts of iodine in the diet can cause hyperthyroidal effects
414 such as anxiety and heart palpitations. Hence, health authorities recommend a diet balanced in iodine.
415 Therefore concerns are often raised when seaweeds are included in the diet. The variation in the
416 iodine content in dietary seaweeds is, however, extremely large and can vary three orders of
417 magnitude. The red seaweed laver (*Pyropia* spp.) used for making *nori* is a low as 1 µg/g whereas
418 *konbu* (*Saccharina japonica*) ranges up to 5,000 µg/g (van Netten et al., 2000; Teas et al., 2004; Holdt
419 & Kraan, 2011), all numbers referring to dry weight. Another popular seaweed dulse (*Palmaria*
420 *palmata*) is also very low in iodine, about 5 µg/g (Mouritsen et al., 2013).

421

422 Many of the brown seaweeds studied in the present paper are also very high in iodine (calculated
423 from Table 2), e.g. the Laminariales typically 1,000-3000 µg/g, and the Fucales typically 200-300
424 µg/g, both with reference to dry weight. For good reason, the health authorities in many countries are
425 therefore hesitant to approve on foodstuff including brown seaweeds. In European countries where
426 these seaweeds are not part of the food culture, they have to be declared and approved as novel food.
427 This leads to problems for harvesters and small companies that are embarking on setting up seaweed
428 businesses. It may in this context be interesting to turn to food cultures, e.g., the Japanese, that
429 consume large amounts of seaweeds. In a recent study, the average intake of iodine in Japan was
430 estimated to be 1,000-3,000 µg per day for an average person, and most of this comes from seaweeds
431 in the diet (Zava & Zava, 2011), e.g., via common *dashi*-based soups like miso soup. Hence the intake
432 is often more than an order of magnitude larger than recommended. Turning to the types of *dashi*
433 investigated in this paper, a bowl of soup (3 dL) can contain more than 2,000 µg if made from
434 Laminariales and more than 300 µg if made from the Fucales.

435

436 The mineral contents in the bull kelp salt precipitate, 37Nl, is very high. However, it is striking that
437 potassium content is almost a factor of 20 larger than that of sodium.

438

439

440 *3.4 Sensory characteristics*

441 Table 4 lists the mean values for the 16 samples that were subjected to sensory analysis. The ANOVA
442 show that 18 of the 23 descriptors varied significantly across the 16 samples. The mean values for
443 the 5 descriptors (white fish (O and F), rubber (O), crab-like (F), raw potato (F)) that did not contribute
444 to discriminate samples are not reported in Table 4, and they are omitted from further analysis. Close
445 scrutiny of the mean values and confidence intervals reveals that all 16 samples are significantly
446 different from each other based on the sensory descriptors. The results of the A-PLSR show that 5
447 dimensions are the optimal solution to extract all of the systematic variance in the data set. The 5
448 dimensions explain 73% of the variance in the data. An evaluation of the stability and the precision
449 in prediction of the individual descriptors is obtained from the A-PLSR by extracting the Root Mean
450 Square Error of Calibration (RMSEC) at the optimal number of dimensions. Those are listed in Table
451 4 in the penultimate row. RMSEC and confidence intervals from uni-variate ANOVA are not directly
452 mathematically related (Johansen et al., 2008). RMSEC is a good measure for predictive ability of
453 the independent variables and will be used for this purpose in Sec. 3.5. Here it suffices to state that
454 colour intensity and fatty after mouthfeel are the two descriptors that are least stable in prediction.
455 Figs. 9a and b show correlation loading plots from the A-PLSR.

456

457 Based on the correlation plots in Figs. 9a and b we have made the following more remarkable
458 observations. The different types of *konbu* are grouped together on the right-hand side of Fig. 9 along
459 dimension 1. They are distinguished by the highest perceived umami intensity. The bull kelp salt
460 precipitate (37Nl) is located in the lower part of dimension 2 and characterized as the most salty, sour,
461 and viscous *dashi* sample. In the left part of Fig. 9a along dimension 1 are samples that are perceived
462 high in roasted, bitter, astringent, and with the highest colour intensity. The two samples that are
463 distinct along dimension 2 are 13Sl, sugar kelp and 32Fv bladder wrack, both from Lillebælt in
464 Danmark, and the two only Danish seaweeds. They are characterized by having the lowest umami
465 taste among the tested *dashis* (cf. Table 4). 15Up, the *wakame* from Brittany, is separated from the
466 others in dimension 3 (Fig. 9b). It is characterized by being fishy. The bull kelp sample (01Nl) is the
467 most turbid sample, which is separated out in dimension 4.

468

469 Sensory characteristics was investigated by PLSR. The results of the PLSR shows that 5 dimensions
470 is the optimal solution to extract all of the systematic variance in the data set. The 5 dimensions
471 explain 54% of the variance in the data. That is a substantial decrease in explained variance, compared
472 to the 74%, when considering the sensory data alone.

473

474 Initially RMSEC was calculated for sensory variables in the A-PLSR analysis based on the design
475 matrix. To characterize all the systematic variance in the sensory data, 5 dimension were necessary.
476 Table 4 lists the RSMEC in the penultimate line, using sensory replicates as cross-validation segments
477 with the optimal number of dimensions. In the last line the RMSEC for prediction of sensory variables
478 from the chemical measurements are listed. Scrutiny of the RMSEC for the two different models
479 reveals that the sensory variables that are less well predicted from the analysis by HPLC and GC are
480 colour intensity, salty taste, astringent and bitter taste. The remainder of the sensory descriptors are
481 well predicted by HPLC and GC measurements. It is surprising that salty taste is not well predicted,
482 as it should arise mainly from sodium and potassium. Odour-induced saltiness enhancement
483 (Lawrence et al., 2011) is a possible reason for the poor prediction of salty taste. The likely origin of
484 the sensory properties of colour intensity, astringent and bitter are all compounds or physical
485 phenomena that were not directly measured by the chosen methods.

486

487 The distribution of samples and variables is show in Figs. 10a and b. Surprisingly, dimensions 1
488 explains less variance (9%) in the sensory data compared to dimension 2 (19%), see Fig. 10a. The
489 majority of the reason for this lies in large differences in the chemical measurements for particularly
490 sample 15Up, the wakame from France, whereas that sample does not distinguish itself in the sensory
491 measurement (see Fig. 9a). The first dimension largely separates this sample from the other 15. It is
492 characterized by strong fishy taste, that may be linked to the high concentration of the many aroma
493 compounds in the right part of dimension 1.

494

495 Dimension 2 groups the Japanese *konbu* in the lower part, characterized by high umami, sweetness,
496 and high viscosity. That must be linked to high glutamate. To some degree the *konbu* samples also
497 have mushroom aroma above average. Table 4 shows that sample 25Sj in particular has the highest
498 mushroom aroma. The *konbu* samples have a high concentration of 1-octen-3-one, a compound with

499 a distinct mushroom taste (cf. also Table 3 for verification). The upper part of dimension 2 contain
500 the two Danish seaweeds 32Fv (bladderwrack) and 13Sl (sugar kelp). Both are low in umami, but
501 high in roasted taste and aroma, and has an above average intensity of bitterness and astringency. The
502 roasted notes arise from beta-cyclocitral, 2- and 3-methylbutanal, and beta-ionone, even though the
503 compounds alone do not possess these properties on (cf. Table 3). Dimensions 3 and 4 (Fig. 10b)
504 separate the samples 01Nl, bull kelp, and 37Nl, the bull kelp salt. Both of them have a high turbidity,
505 but also high concentration of (E)-3-octen-2-one. The bull kelp salt is also characterized by high
506 concentration of 1-heptanol, 1-octanol, and 1-heptanol, and an extremely high concentration of
507 potassium (cf. also Table 2).Dimension 5 (not shown) indicates a group of compounds 2,3-
508 butanedione, 2-pentanone, 3 and 2-methylbutanal, that are high in three of the samples originating
509 from Vancouver Island, not only the bull kelp (01Nl) but also the North Pacific *konbu*, *Laminaria*
510 *setchelli* (03Ls), and to a lesser degree the macro kelp (02Mp) also has some content of these
511 compounds.

512

513 **4. Conclusion**

514 The primary aim of the present study was to investigate the umami potential of a series of brown
515 seaweeds that are commonly used as foodstuff around the world. The umami taste is elicited by free
516 Glu (and to some extent of free Asp). Synergy in the umami taste can be induced by the simultaneous
517 presence of free nucleotides, such as inosinate. We did not measure free nucleotides in the present
518 study but it is known that only few seaweeds contain any appreciable amounts of inosinate, with laver
519 (*Pyropia* spp.) used for *nori* being a notorious exception (Ninomiya, 2002). We have found that *konbu*
520 has the largest contents of free Glu, with an appreciable variation over the various Japanese
521 subspecies of *Saccharina japonica*. The related species sugar kelp (*Saccharina latissimi*) together
522 with other members of the Laminariales have low levels of free Glu, but in comparison to *konbu*
523 fairly high levels of the sweet, free amino acid Ala.

524

525 The perception of umami does, however, not only depend on the glutamate content but also on how
526 other tastes and volatile aroma compounds influence the taste experience. This is indirectly
527 demonstrated in Fig. xxx where the perceived intensity of umami is plotted against the measured
528 contents of glutamate. In addition, the sensory data are substantially worse predicted by the chemical

measurements compared to the systematic variance that is extracted when analysing the sensory data alone. This indicates that there are compounds that we have not analysed for that cause bitterness, colour, and astringency.

The results presented in the present paper can form a basis for a more informed use of brown seaweeds as a flavour-giver to foodstuff and to which extent the different species can contribute to umami taste, e.g., in a soup broth and as a cooking or marinating medium for other ingredients, in particular vegetables, that have very little umami on their own (Maga, 1983; Mouritsen and Styrbæk, 2014). The use of brown seaweeds to flavour food may therefore, via the rising phycogastronomy (Mouritsen et al., 2018), possibly help to encourage a larger intake of greens, stimulate appetite, regulate food intake, and hence improve nutrition and health (Kondoh et al., 2009; Mouritsen, 2012; Cornish et al., 2015). The results of the paper may also be used to evaluate iodine load of seaweed products and act as a guide in the use of different seaweeds as a salt substitute with a high K/Na ratio (Yamaguchi and Takahashi, 1984). The particular salt precipitate from the brown seaweed bull kelp mentioned and studied in this paper is of particular interest as a salt substitute with a very low Na/K-ratio and a sour, salty, and bitter taste.

Acknowledgements

The work by OGM, ALH, and MBF is supported by the Nordea-fonden via a centre grant to *Taste for Life.* Louis Druehl is thanked for a generous gift of a salt precipitate from *Nereocystis leutkeana* and samples of *Macrocystis*, *Nereocystis,* and *Laminaria* harvested in Barkley Sound, Vancouver Island. Anita Dietz is acknowledged for providing samples of Danish seaweeds. Koji Kinoshita is thanked for interpretation of the Japanese description on the package of some commercial seaweeds.

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661

662 **Tables**

663 **Table 1**

664 Samples investigated in the present study and their origin.

665

666 Table 2

667 Concentrations of free amino acids, iodine, sodium, and potassium in 3 dried seaweed samples
668 extracted in *dashi* (Cf. Table 1).

669 Footnote:

670 Free amino acids in units of mg/100mL; iodine in units of µg/L; sodium and potassium in units of
671 mg/L. The uncertainty in the measured values is typically 15%. Analysis were carried out in
672 quadruplicates. p-values and 95% confidence intervals (CI95%) are from ANOVA. Ala: alanine; Arg:
673 arginine; Asn: asparagine; Asp: aspartic acid (aspartate); Cys: cysteine, Gln: glutamine; Glu: glutamic
674 acid (glutamate); Gly: glycine; His: histidine; Ile+Leu: isoleucine+leucine; Lys: lysine; Met:
675 methionine; Phe: phenylalanine; Pro: proline; Ser: serine; Thr: threonine; Trp: tryptophan; Tyr:
676 tyrosine; Val: valine.

677

678 **Table 3**

679 Contents of identified volatile compounds (relative concentrations) in *dashies* extracted from 16
680 selected dried seaweed samples (cf. Table 1).

681 Footnote:

682 Values are means of triplicate determinations except for samples 23Sj, 25Sj, and 26Sj (duplicate).
683 Only volatiles with significant differences between samples (determined via ANOVA) are included.

684

685 **Table 4**

686 Sensory characteristics of of *dashies* extracted from 16 selected dried seaweed samples (cf. Table 1).

687 Footnote:

688 Mean rating on a scale 1-10 (over panellists and replicates) of intensity for all significant sensory
689 descriptors for the 16 samples of *dashis* subjected to descriptive analysis. p-values and 95%
690 confidence intervals (CI95%) are from ANOVA. Abbreviations in labels refer to: orthonasal odour
691 (O), turbidity/visual appearance (A), taste (T), flavour i.e., retronasal odour perception (F), mouthfeel
692 (M), and after mouthfeel (AF).

693

694 **Figures**

695 **Fig. 1.** A-PLSR analysis of HPLC data. (a): Correlation loading plot of dimensions 1 (25%) and 2
696 (10%). (b): Correlation loading plot of dimensions 3 (6%) and 4 (5%). The plots reveal the
697 interrelationship between variables and samples; *dashi* samples (in blue) and AA/ions (in red). For
698 clarity of the figure, labels for less important samples are abbreviated or omitted. Confer table 1 for
699 sample names.
700
701 **Fig. 2.** A-PLSR analysis of GC data. (a): Correlation loading plot of dimensions 1 (58%) and 2 (9%).
702 (b): Correlation loading plot of dimensions 3 (4%) and 4 (3%). The plots reveal the interrelationship
703 between variables and samples; *dashi* samples (in blue) and aroma compounds (in red). For clarity
704 of the figure, labels for less important compounds and samples are abbreviated or omitted. Confer
705 table 3 for compound names.
706
707 **Fig. 3.** A-PLSR analysis of HPLC data, limited to the samples that were also subjected to GC and
708 sensory analysis. Correlation loading plot of dimensions 1 (24%) and 2 (10%); *dashi* samples (in
709 blue) and AA/ions (in red).
710
711 **Fig. xxx.** Contents of free glutamate (xxx), free aspartate (xxx), and alanine (xxx) for all samples
712 arranged according to magnitude of free glutamate content.
713
714 **Fig. xxx.** Contents of iodine for all samples arranged according to magnitude of iodine contents.
715
716 **Fig. 9.** A-PLSR analysis based on sensory description. (a): Correlation loading plot of dimensions 1
717 (34%) and 4 (22%). (b): Correlation loading plot of dimensions 3 (11%) and 4 (6%); *dashi* samples
718 (in blue) and sensory descriptors (in green).
719
720 **Fig. 10.** A-PLSR analysis; X: HPLC; GC, Y: Sensory analysis. (a): Correlation loading plot of
721 dimensions 1 (9%) and 2 (19%). (b): Correlation loading plot of dimensions 3 (12%) and 4 (8%).;
722 *dashi* samples (in blue), aroma compounds/AA/ions (in two red colors) and sensory descriptors (in
723 green). Less relevant variables are omitted for clarity.

724

725 **Fig. xxx.** Plot of perceived umami intensity from the sensory analysis as a function of amount of free
726 glutamate measured by HPLC for all 16 *dashi* samples subjected to sensory analysis.

table

Click here to access/download;table;Tables-rev.xlsx

Table 1

Sample name	Common name	Species	Origin
01Nl	Bull kelp	*Nereocystis leutkeana*	Bamfield, Vancouver Island
02Mp	Macrokelp	*Macrocystis pyrifera*	Bamfield, Vancouver Island
03Ls	North Pacific konbu (2016a)	*Laminaria setchellii*	Bamfield, Vancouver Island
04Ls	North Pacific *konbu* (2016b)	*Laminaria setchellii*	Bamfield, Vancouver Island
05Lg	North Pacific *konbu* (2000)	*Laminaria groenlandica*	Bamfield, Vancouver Island
06Ld	Atlantic *konbu*	*Laminaria digitata*	Grindavik, Island
07Ld	Oarweed	*Laminaria digitata*	Lillebælt, Denmark
08Lh	Tangleweed	*Laminaria hyperborea*	Stykkishólmur, Iceland
09Sl	Sugar kelp	*Saccharina longicrusis*	Maine, USA
10Sl	Sugar kelp	*Saccharina latissima*	Grindavik, Iceland
11Sl	Sugar kelp	*Saccharina latissima*	Storebælt, Denmark
12Sl	Sugar kelp	*Saccharina latissima*	Samsø, Denmark
13Sl	Sugar kelp	*Saccharina latissima*	Lillebælt, Denmark
14Up	*Wakame*	*Undaria pinnatifida*	Shimane, Japan
15Up	*Wakame*	*Undaria pinnatifida*	Brittany, France
16Ae	Winged kelp	*Alaria esculenta*	Stykkishólmur, Iceland
17Ae	Winged kelp	*Alaria esculenta*	Grindavik, Iceland
18Sj	*Hidaka-konbu* (Daichu Kahomotsu Hidaka)	*Saccharina japonica*	Hokkaido, Japan
19Sj	*Hidaka-konbu* (Tokon Hidaka)	*Saccharina japonica*	Hokkaido, Japan
20Sj	*Rishiri-konbu (* Akafundo)	*Saccharina japonica*	Hokkaido, Japan
21Sj	*Rishiri-konbu* (Ao Fundo)	*Saccharina japonica*	Hokkaido, Japan
22Sj	*Rishiri-konbu* (Matsumaya)	*Saccharina japonica*	Hokkaido, Japan
23Sj	*Rishiri-konbu (* Fukui district)	*Saccharina japonica*	Hokkaido, Japan
24Sj	*Raushu-konbu*	*Saccharina japonica*	Hokkaido, Japan
25Sj	*Raushu-konbu* (Fukuoka)	*Saccharina japonica*	Hokkaido, Japan
26Sj	*Ma-konbu (* Fukuoka)	*Saccharina japonica*	Hokkaido, Japan
27Sj	*Ma-konbu* (Sakai)	*Saccharina japonica*	Hokkaido, Japan
28Pp	Sea palm	*Postelsia palmaeformis*	California, USA
29He	Thongweeed	*Himanthalia elongata*	Brittany, France
30Eb	*Arame*	*Ecklonia bicyclis*	Chiba, Japan
31Sf	*Hijiki*	*Sargassum fusiforme*	Japan
32Fv	Bladderwrack	*Fucus vesiculosus*	Lillebælt, Denmark
33Fsp	Spiraled wrack	*Fucus spiralis*	Lillebælt, Denmark
34Fe	Rockweed	*Fucus evanescens*	Amager, Denmark
35Fse	Serrated wrack	*Fucus serratus*	Lillebælt, Denmark
36Cf	Sea lace	*Chorda filum*	Samsø Bælt, Denmark
37Nl	Bull kelp salt	*Nereocystis leutkeana*	Bamfield, Vancouver Island

Table 2

Sample name	Subjected to GC and sensory analysis	Gly	Ala	Ser	Pro	Val	Thr	Asp	Lys	Glu	Met	His	Phe	Arg	Tyr	Cys2	Ile+Leu	[I]	[Na]	[K]
01Nl	yes	1.5	12	0.10	1.9	0.43	2.4	0.26	0.14	2.5	0	0.065	0.60	0.33	0.10	0.010	0.59	670	1000	1800
02Mp	yes	0.22	8.3	0.0094	0.28	0.16	1.0	0.048	0.015	1.0	0	0.015	0.28	0.063	0.040	0.0025	0.30	3800	850	1800
03Ls	yes	0.31	15	0.022	0.47	0.21	1.2	0.17	0.033	0.59	0.010	0.020	0.53	0.21	0.043	0.0050	0.27	2500	450	680
04Ls		0.35	11	0.018	0.44	0.23	1.2	0.13	0.023	0.49	0.0050	0.028	0.42	0.16	0.038	0	0.27	600	490	1000
05Lg		0.075	4.2	0.0085	0.10	0.040	0.90	0.065	0.015	0.31	0.015	0.0050	0.14	0.083	0.013	0	0.050	13000	520	670
06Ld		0.38	11	0.018	0.49	0.15	1.3	0.10	0.025	1.1	0.0050	0.025	0.27	0.12	0.035	0.0075	0.19	4400	940	1300
07Ld		0.54	14	0.019	0.70	0.22	2.3	0.060	0.025	0.56	0	0.020	0.35	0.078	0.063	0.015	0.25	3100	620	1400
08Lh		1.0	12	0.011	1.3	0.063	1.7	0.25	0.015	1.5	0.010	0.020	0.25	0.30	0.018	0	0.045	6800	1000	1800
09Sl		0.68	8.9	0.0064	0.87	0.070	0.91	0.050	0.008	1.8	0	0	0.18	0.063	0.005	0	0.035	2600	1000	2100
10Sl	yes	0.39	3.9	0.0037	0.49	0.29	0.62	0.055	0.010	0.86	0	0.0075	0.14	0.068	0.015	0.0050	0.19	4400	690	290
11Sl		0.070	1.2	0.0030	0.085	0.038	0.08	0.023	0.008	0.19	0	0.0025	0.13	0.025	0	0.0075	0.020	2400	440	500
12Sl		0.081	0.64	0.0031	0.10	0.045	0.043	0.028	0.005	0.12	0	0.013	0.14	0.033	0.008	0.0075	0.020	480	480	220
13Sl	yes	0.37	8.8	0.013	0.47	0.36	1.0	0.055	0.020	1.1	0.020	0.028	0.49	0.073	0.048	0.010	0.26	1600	460	690
14Up		0.77	15	0.055	1.0	0.12	3.1	0.28	0.078	3.9	0.040	0.045	0.70	0.34	0.070	0.0050	0.62	110	620	1500
15Up	yes	0	0	0.0064	0.010	0	0	0.005	0.005	0.015	0	0	0	0.005	0	0	0	60	1200	42
16Ae	yes	1.1	7.8	0.0077	1.4	0.088	0.67	0.068	0.013	0.33	0	0.0050	0.35	0.083	0.078	0.0050	0.11	160	1600	340
17Ae		0.24	7.4	0.014	0.24	0.065	1.2	0.090	0.015	1.2	0	0.010	0.15	0.12	0.008	0.0075	0.045	300	920	1000
18Sj	yes	0.84	1.0	0.011	1.0	0.043	0.13	0.058	0.013	18	0	0.010	0.12	0.075	0	0.018	0.050	5400	710	720
19Sj		0.77	1.5	0.010	1.0	0.045	0.18	0.055	0.013	4.3	0	0.010	0.11	0.070	0.005	0.0075	0.040	1700	690	1300
20Sj		0.92	0.38	0.014	1.2	0.087	0	0.018	0.015	28	0	0.0050	0.49	0.023	0.43	0.27	0.060	6200	630	2000
21Sj		0.69	0.53	0.012	0.87	0.055	0.12	0.048	0.018	31	0	0.0050	0.20	0.055	0.020	0.0075	0.085	3100	540	970
22Sj		0.31	0.18	0.011	0.42	0.010	0.040	0.018	0.013	11	0	0.0075	0.30	0.020	0.005	0.015	0.055	4100	600	980
23Sj	yes	0.87	0.35	0.033	1.1	0.043	0.090	0.025	0.045	23	0	0.015	0.78	0.028	0.040	0	0.075	4200	480	960
24Sj		0.84	0.42	0.034	1.0	0.050	0.090	0.030	0.048	27	0.010	0.030	1.2	0.035	0.053	0	0.085	2600	530	1700
25Sj	yes	0.57	0.37	0.012	0.72	0.038	0	0.020	0.015	23	0.010	0.0050	0.28	0.020	0.030	0.0050	0.030	4300	530	1900
26Sj	yes	1.4	0.47	0.011	1.78	0.050	0.093	0.030	0.013	37	0.015	0.010	0.23	0.035	0.0025	0	0.11	5700	560	1400
27Sj	yes	0.33	0.42	0.0071	0.42	0.053	0.040	0.023	0.010	28	0	0.010	0.19	0.028	0.0050	0.0025	0.075	5100	800	1900
28Pp	yes	0.45	5.7	0.016	0.57	0.085	1.0	0.13	0.020	2.2	0	0.015	0.20	0.16	0.0325	0.018	0.045	1100	1100	820
29He		0.12	1.3	0.0066	0.15	0.10	0.34	0.023	0.010	0.55	0	0.0025	0.10	0.02	0.0275	0.0050	0.035	110	1300	2600
30Eb	yes	1.1	5.5	0.0041	1.3	0.028	0.56	0.063	0.008	0.81	0	0.0025	0.10	0.08	0.0025	0.010	0.030	3000	890	3200
31Sf		0.048	0.12	0.0044	0.053	0.015	0	0.010	0.0067	0.073	0	0.030	0.030	0.018	0	0	0	490	210	680
32Fv	yes	0.14	2.0	0.012	0.18	0.078	1.1	0.16	0.015	1.4	0	0.048	0.37	0.20	0.025	0	0.060	950	720	650
33Fsp		0.21	0.8	0.013	0.25	0.070	0.77	0.19	0.018	3.1	0	0.028	0.33	0.24	0.040	0	0.10	240	710	650
34Fe		0.073	1.9	0.011	0.088	0.073	0.89	0.16	0.015	1.8	0	0.020	0.25	0.19	0.030	0.0025	0.060	1700	480	570
35Fse		0.24	1.4	0.010	0.30	0.068	0.79	0.14	0.010	1.4	0	0.023	0.34	0.18	0.033	0.010	0.070	580	510	1000
36Cf		0.10	0.59	0.0029	0.11	0.033	0.54	0.033	0.005	0.14	0	0.0025	0.060	0.038	0.010	0.013	0.020	1400	320	590
37Nl	yes	0.49	3.5	0.025	0.64	0.14	0.70	0.16	0.033	0.84	0	0.0075	0.13	0.20	0.038	0	0.16	1400	470	7900
p-value		1E-08	7E-34	2E-05	4E-10	3E-11	3E-14	1E-03	3E-06	5E-32	4E-12	2E-06	2E-05	8E-04	4E-15	7E-01	4E-22	9E-40	3E-23	6E-32
Significance level		***	***	***	***	***	***	**	***	***	***	***	***	***	***	NS	***	***	***	***
Confidence intervals		0.39	2.2	0.021	0.47	0.086	0.59	0.10	0.028	5.1	0.0069	0.016	0.28	0.12	0.018	n/a	0.088	1000	210	620
RMSEC - 4reps HPLC from design three factors - 38 samples		0.48	3.7	0.025	0.58	0.11	0.72	0.11	0.033	7.0	0.0092	0.019	0.34	0.13	0.025	n/a	0.12	2300	340	1300
RMSEC - 4reps HPLC from design three factors 16 samples		0.62	3.6	0.039	0.73	0.15	0.75	0.12	0.051	7.2	0.0098	0.023	0.33	0.15	0.03	n/a	0.16	1400	270	1900

Table 3

Compound	KI	Identification[1)]	Sample name 01Nl	02Mp	03Ls	10Sl	13Sl	15Up	16Ae	18Sj	23Sj	25Sj	26Sj	27Sj	28Pp	30Eb	32Fv	37Nl	Odour descriptor[2)]
Alcohols																			
1-penten-3-ol	1179	MS, KI	0.046	0.0093	0.014	0.012	0.063	0.55	0.087	0.010	0.0041	0.0029	0.00029	0.0065	0.023	0.010	0.097	0.012	pungent, green
1-pentanol	1274	MS, KI, auth	0.089	0.015	0.012	0.028	0.024	0.55	0.043	0.027	0.021	0.043	0.014	0.034	0.065	0.056	0.047	0.053	pungent, fermented
(Z)-2-penten-1-ol	1342	MS, KI, auth	0.072	0	0.015	0	0.10	0.52	0.072	0.0071	0	0	0	0	0.067	0.042	0.16	0.046	green
1-hexanol	1371	MS, KI, auth	0.093	0.062	0.065	0.093	0.090	0.34	0.19	0.085	0.029	0.081	0.051	0.075	0.14	0.13	0.088	0.32	fusel, oily
1-octen-3-ol	1464	MS, KI, auth	0.039	0.012	0.016	0.071	0.060	0.42	0.17	0.079	0.052	0.053	0.042	0.089	0.12	0.053	0.16	0.029	mushroom
1-heptanol	1470	MS, KI, auth	0.14	0.11	0.055	0.075	0.083	0.26	0.13	0.096	0.086	0.11	0.078	0.11	0.27	0.17	0.11	0.22	musty, leafy
1,7-octadien-3-ol	1520	MS	0	0.0034	0.0049	0.0085	0.004	0.52	0.027	0	0	0	0	0	0.0017	0	0.038	0	
1-octanol	1572	MS, KI, auth	0.14	0.047	0.038	0.039	0.054	0.082	0.047	0.10	0.041	0.056	0.042	0.085	0.39	0.045	0.063	0.30	waxy, green
(E)-2-octen-1-ol	1631	MS, KI	0.049	0.0070	0.016	0.026	0.039	0.45	0.096	0.049	0.017	0.030	0.038	0.064	0.18	0.037	0.16	0.12	green, citrus
2,7-octadien-1-ol	1701	MS	0.037	0.022	0.021	0.019	0.033	0.53	0.11	0.023	0.020	0.022	0.014	0.022	0.12	0.029	0.11	0.069	
(Z)-2-nonen-1-ol	1730	MS	0.0010	0	0	0.00036	0	0	0.011	0.0082	0.20	0.33	0.31	0.49	0.014	0.0029	0.00072	0.0026	waxy, melon
Aldehydes																			
Butanal	879	MS, KI	0.10	0.025	0.036	0.039	0.11	0.47	0.11	0.099	0.032	0.046	0.025	0.037	0.067	0.067	0.051	0.12	pungent, green
2-methylbutanal	913	MS, KI, auth	0.19	0.13	0.12	0.16	0.36	0.028	0.21	0.067	0.038	0.059	0.028	0.11	0.056	0.072	0.059	0.098	musty, cocoa, coffee
3-methylbutanal	917	MS, KI, auth	0.28	0.098	0.099	0.31	0.25	0.065	0.13	0.10	0.054	0.11	0.027	0.17	0.079	0.071	0.063	0.12	dry, green, chocolate
Pentanal	979	MS, KI, auth	0.14	0.028	0	0.053	0.060	0.44	0.084	0.083	0.032	0.050	0.019	0.064	0.14	0.071	0.052	0.20	fermented, bready
(E)-2-butenal	1038	MS, KI	0.062	0	0.027	0.0077	0.011	0.47	0.0079	0.039	0.089	0.12	0.105	0.018	0.13	0.0035	0.0056	0.14	flower
2-methyl-(E)-2-butenal	1093	MS, KI	0.16	0.051	0.035	0.13	0.049	0.50	0.050	0.038	0.066	0.078	0	0.061	0.034	0.024	0.0053	0.052	pungent, green
(E)-2-pentenal	1135	MS, KI	0.034	0.011	0.014	0.012	0.016	0.55	0.051	0.015	0.038	0.013	0	0.0080	0.021	0.014	0.010	0.036	pungent, green
2-methyl-2-pentenal	1164	MS, KI	0.025	0	0	0.00017	0.0053	0.54	0.011	0.0039	0	0	0	0	0.010	0.0059	0.0031	0.014	green, grassy
Heptanal	1191	MS, KI, auth	0.14	0.057	0.062	0.062	0.082	0.43	0.080	0.10	0.075	0.088	0.060	0.12	0.14	0.073	0.067	0.16	fatty, green
(E)-2-hexenal	1224	MS, KI, auth	0.064	0.010	0.063	0.013	0.034	0.54	0.027	0.015	0.0053	0.060	0	0.0083	0.014	0.012	0.023	0.032	green, leafy
(Z)-4-heptenal	1255	MS, KI	0.050	0.00017	0.0090	0.0063	0.040	0.55	0.029	0.014	0.045	0.011	0	0.0039	0.036	0.0065	0.0089	0.033	fatty, green
Octanal	1305	MS, KI, auth	0.19	0.071	0.077	0.057	0.076	0.14	0.075	0.10	0.087	0.084	0.073	0.12	0.35	0.063	0.069	0.15	citrus
(E,Z)-2,4-heptadienal	1508	MS, KI, auth	0.025	0.0062	0.00068	0.00068	0.00028	0.55	0.012	0.0088	0	0	0	0	0.067	0.0016	0.0039	0.072	fried
Benzaldehyde	1540	MS, KI, auth	0.14	0.13	0.16	0.10	0.13	0.36	0.17	0.097	0.099	0.12	0.048	0.10	0.140	0.068	0.044	0.14	almond
(E)-2-nonenal	1550	MS, KI, auth	0.14	0.030	0.055	0.039	0.037	0.15	0.045	0.084	0.14	0.32	0.27	0.36	0.095	0.039	0.027	0.11	cardboard
2,5-dimethyl-benzaldehyde	1754	MS, KI	0.10	0.11	0.12	0.10	0.12	0.44	0.12	0.088	0.049	0.062	0.032	0.091	0.094	0.051	0.094	0.072	
Alkane																			
7-oxabicyclo[2.2.1]heptane	1143	MS	0.11	0	0.0038	0.018	0.064	0.53	0.054	0.021	0	0.011	0	0.0060	0.057	0.017	0.014	0.065	
Amine																			
Trimethylamine	616	MS, KI	0.014	0.000	0.0078	0.0085	0	0	0.015	0.078	0.0036	0.0062	0.0021	0	0.011	0.015	0.49	0.0036	fishy
Carotenoid derived																			
2,2,6-trimethylcyclohexanone	1329	MS, KI	0.079	0.030	0.042	0.068	0.36	0.26	0.24	0.061	0.031	0.021	0.025	0.048	0.054	0.16	0.16	0.024	pungent, thujonic
1,6-dimethylhepta-1,3,5-triene	1506	MS	0.064	0.011	0.028	0.039	0.11	0.49	0.10	0.034	0.014	0.038	0.0064	0.031	0.047	0.054	0.034	0.033	
Beta-cyclocitral	1641	MS, KI	0.13	0.12	0.11	0.038	0.42	0.10	0.099	0.023	0.024	0.0067	0.032	0.039	0.035	0.037	0.27	0.013	tropical, saffron
4-oxoisophorone	1714	MS, KI	0.017	0.29	0.019	0.020	0.040	0.47	0.10	0.010	0.018	0.013	0	0.027	0.037	0.025	0.030	0.025	musty, woody
Beta-ionone	1967	MS, KI	0.12	0.056	0.075	0.040	0.43	0.16	0.14	0.028	0.0075	0	0.013	0.0073	0.028	0.038	0.18	0.0065	floral, woody
Ketones																			
2-butanone	906	MS, KI, auth	0.096	0.091	0.079	0.13	0.10	0.31	0.16	0.081	0.055	0.066	0.046	0.11	0.10	0.16	0.21	0.23	acetone
2-pentanone	983	MS, KI, auth	0.15	0.080	0.22	0.10	0.085	0.36	0.19	0.074	0.031	0.024	0.011	0.085	0.064	0.10	0.072	0.17	fruity, winey
2,3-butanedione	985	MS, KI, auth	0.35	0.029	0.34	0.042	0.043	0.041	0.037	0.026	0.022	0.037	0.016	0.041	0.044	0.0089	0.0066	0.090	buttery, caramel
1-penten-3-one	1022	MS, KI	0.097	0.050	0.026	0.021	0.33	0.38	0.16	0.031	0.016	0.014	0	0.011	0.069	0.037	0.043	0.063	peppery, mustard
2-heptanone	1189	MS, KI, auth	0.087	0.038	0.027	0.075	0.17	0.43	0.15	0.061	0.046	0.076	0.035	0.068	0.099	0.14	0.086	0.092	blue cheese
6-methyl-2-heptanone	1251	MS, KI	0.038	0.044	0.027	0.033	0.091	0.46	0.13	0.046	0.042	0.052	0.015	0.038	0.069	0.081	0.13	0.067	camphoreous
2-octanone	1302	MS, KI, auth	0.077	0.079	0.052	0.077	0.12	0.40	0.18	0.088	0.057	0.10	0.048	0.094	0.10	0.15	0.12	0.10	earthy, weedy
1-octen-3-one	1318	MS, KI, auth	0.078	0.013	0.014	0.033	0.064	0.13	0.081	0.10	0.21	0.28	0.17	0.19	0.31	0.044	0.046	0.057	mushroom
(Z)-6-octen-2-one	1348	MS, KI	0.052	0.034	0.012	0.038	0.088	0.48	0.23	0.035	0.0082	0.018	0	0.017	0.040	0.063	0.050	0.047	
6-methyl-5-hepten-2-one	1354	MS, KI, auth	0.11	0.090	0.061	0.090	0.087	0.34	0.14	0.080	0.14	0.11	0.061	0.074	0.18	0.16	0.18	0.13	citrus, green
(E)-3-octen-2-one	1419	MS, KI	0.19	0.024	0.013	0.036	0.037	0.40	0.089	0.059	0.018	0.026	0.014	0.033	0.15	0.064	0.096	0.19	
(E,E)-3,5-octadien-2-one	1587	MS, KI	0.017	0.005	0.0050	0.0075	0.014	0.56	0.070	0.0083	0.0030	0.0049	0.00039	0.0066	0.030	0.014	0.035	0.006	fruity, green
1-phenyl-1-propanone	1747	MS, KI	0.043	0.033	0.0045	0.046	0.055	0.51	0.13	0.054	0.024	0.048	0.0075	0.047	0.11	0.065	0.041	0.067	hawthorne, lilac
1-(3-methylphenyl)-ethanone	1800	MS, KI	0.047	0.043	0.040	0.064	0.087	0.51	0.14	0.057	0.031	0.037	0.033	0.046	0.089	0.077	0.070	0.052	
Lactone																			
γ-nonalactone	2060	MS, KI	0.026	0.044	0.081	0.270	0.16	0.34	0.19	0.11	0.043	0.034	0.055	0.067	0.11	0.073	0.090	0.053	cocnut, fatty

[1)]: MS=Identification by NIST search, KI=Identification confirmed by Kovats Index from literature, auth=Identification confirmed by running an authentic standard

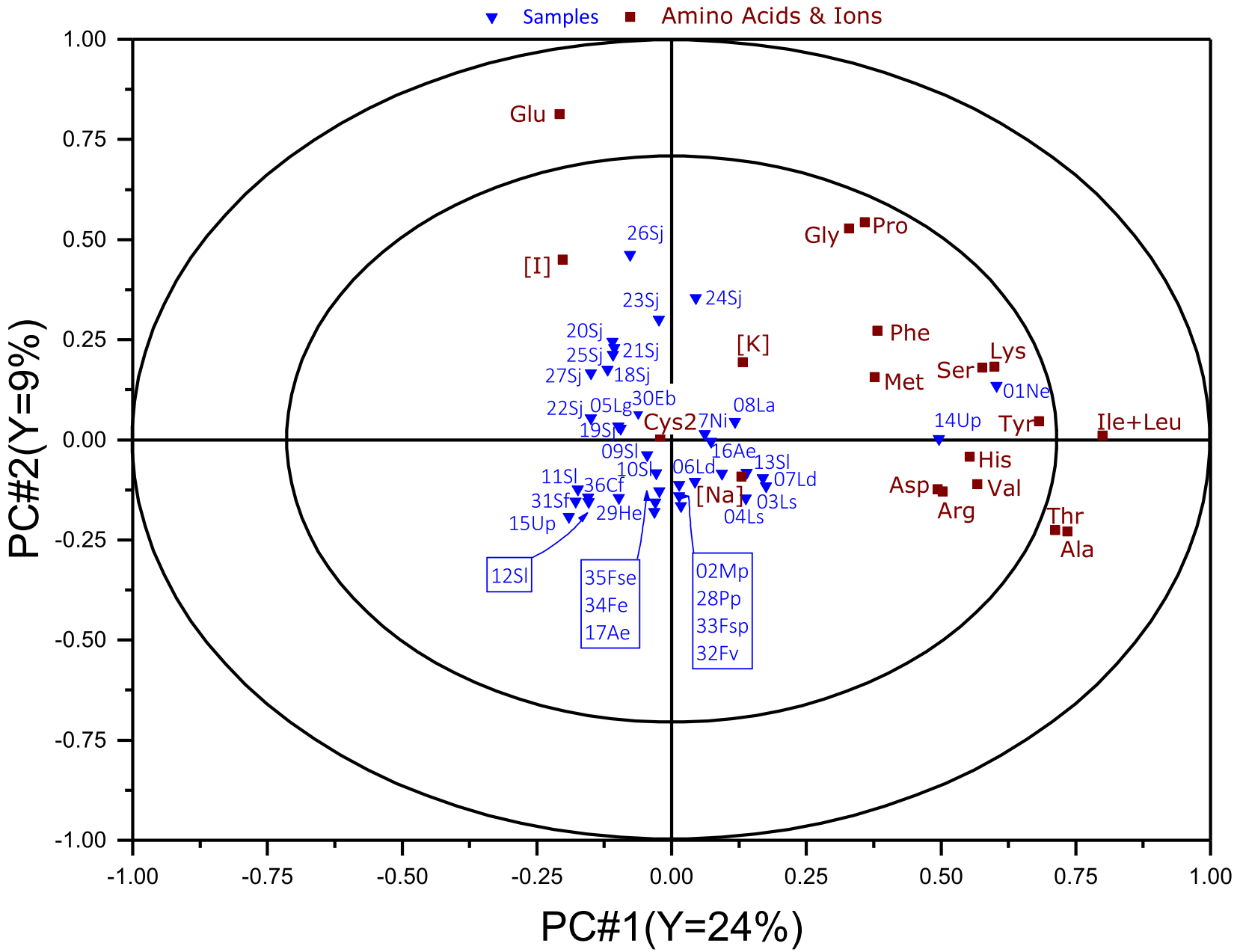
[2)]: Odour descriptors from http://www.thegoodscentscompany.com/

Table 4

Sample name	Turbidity (A)	Color Intensity (A)	Seaweed (O)	Mushroom (O)	Fishy (O)	Tea (O)	Roasted (O)	Roasted (F)	Fishy (F)	Salty (T)	Umami (T)	Sour (T)	Bitter (T)	Sweet (T)	Viscosity (M)	Astringent (AF)	Fatty (AF)	Metallic (AF)
01Nl	6.8	2.9	5.7	5.5	4.0	2.8	2.4	4.2	2.0	6.4	6.5	2.1	2.4	3.4	5.1	3.6	4.0	3.6
02Mp	4.1	7.4	4.6	4.3	2.7	6.2	3.9	5.3	1.8	6.9	5.9	2.3	3.6	2.9	4.8	4.7	3.8	4.3
03Ls	4.4	0.6	5.7	4.7	3.3	2.4	2.7	2.8	1.4	1.7	5.0	0.9	1.9	2.9	3.2	2.7	2.1	3.4
10Sl	3.6	2.5	5.5	5.4	3.6	3.2	4.8	4.5	1.1	1.8	5.5	1.1	2.6	1.9	3.7	3.4	2.5	3.3
13Sl	1.4	4.9	6.7	4.5	4.5	3.8	3.7	4.0	1.5	1.5	4.5	0.8	4.3	2.5	3.1	4.5	2.4	3.6
15Up	1.9	1.3	7.5	3.2	6.3	4.0	2.5	3.5	4.0	6.0	4.8	2.5	3.2	2.0	4.4	4.8	2.8	4.5
16Ae	2.4	2.8	7.3	4.0	4.6	3.4	3.6	3.7	2.6	7.7	5.6	2.2	2.2	2.7	4.4	4.1	4.1	3.8
18Sj	3.9	0.3	6.4	5.2	4.5	2.8	2.2	3.6	1.1	3.2	6.9	1.4	1.8	2.4	4.3	3.1	3.2	3.8
23Sj	1.9	0.3	4.2	5.2	2.7	2.5	2.7	3.4	1.2	2.7	6.9	1.2	1.3	4.3	4.6	3.2	4.2	3.6
25Sj	2.4	0.6	4.8	6.3	1.8	2.9	2.6	3.5	1.1	5.9	7.7	2.1	2.1	3.7	4.3	3.1	3.5	3.9
26Sj	2.3	0.3	3.3	4.9	1.7	2.3	2.4	3.2	1.0	3.9	7.9	1.7	1.4	4.0	4.5	3.2	3.8	3.7
27Sj	2.7	1.4	4.9	5.2	3.2	2.6	2.9	3.8	1.8	6.8	8.0	2.4	2.0	3.2	4.9	3.2	4.1	4.1
28Pp	4.6	5.8	7.5	4.4	5.2	4.0	2.9	4.3	3.1	5.5	5.7	1.8	3.8	2.8	4.2	5.1	3.4	4.5
30Eb	2.2	8.6	6.1	5.8	3.6	3.8	4.8	6.3	2.3	5.9	6.2	2.0	6.7	2.4	4.4	6.0	3.8	4.7
32Fv	1.4	7.8	6.1	4.7	7.2	3.3	3.8	4.1	2.9	2.0	4.6	1.0	2.6	1.6	3.8	4.3	2.8	3.6
37Nl	4.6	1.4	6.5	5.6	4.0	3.2	3.1	3.0	2.8	9.4	6.2	3.8	4.8	2.1	5.4	5.7	4.3	5.8
p-value	p<0.0001	p<0.0001	p<0.0001	p=0.002	p<0.0001	p<0.0001	p<0.0001	p=0.002	p=0.001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p=0.037	p<0.0001	P=0.018	p=0.019
CI95%	0.7	0.4	0.6	0.8	0.8	0.7	0.7	0.7	0.6	0.6	0.7	0.6	0.7	0.6	0.6	0.7	0.8	0.8
RMSEC - Sensory from design 5 factors	1.14	0.45	0.81	0.91	1.12	1.00	0.73	0.74	0.73	0.62	0.89	0.51	0.69	0.72	0.62	0.48	1.11	0.75
RMSEC - Sensory from chemical measurements 5 factors	1.19	2.00	0.99	0.84	1.33	1.04	0.87	0.89	0.69	1.69	0.84	0.64	1.19	0.72	0.63	0.99	1.03	0.79

colour figure

Click here to access/download;colour figure;figure-1.eps



colour figure

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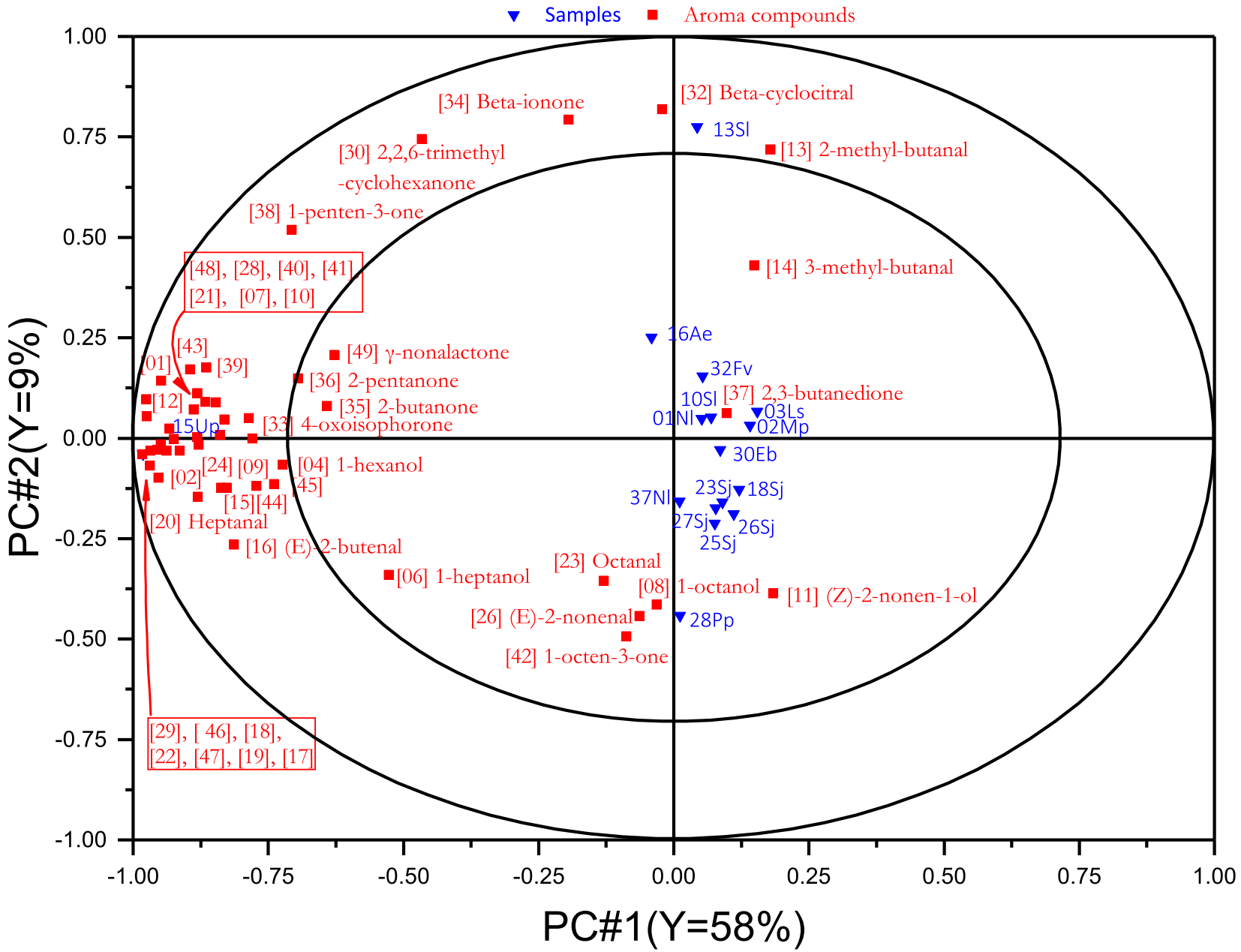

line figure

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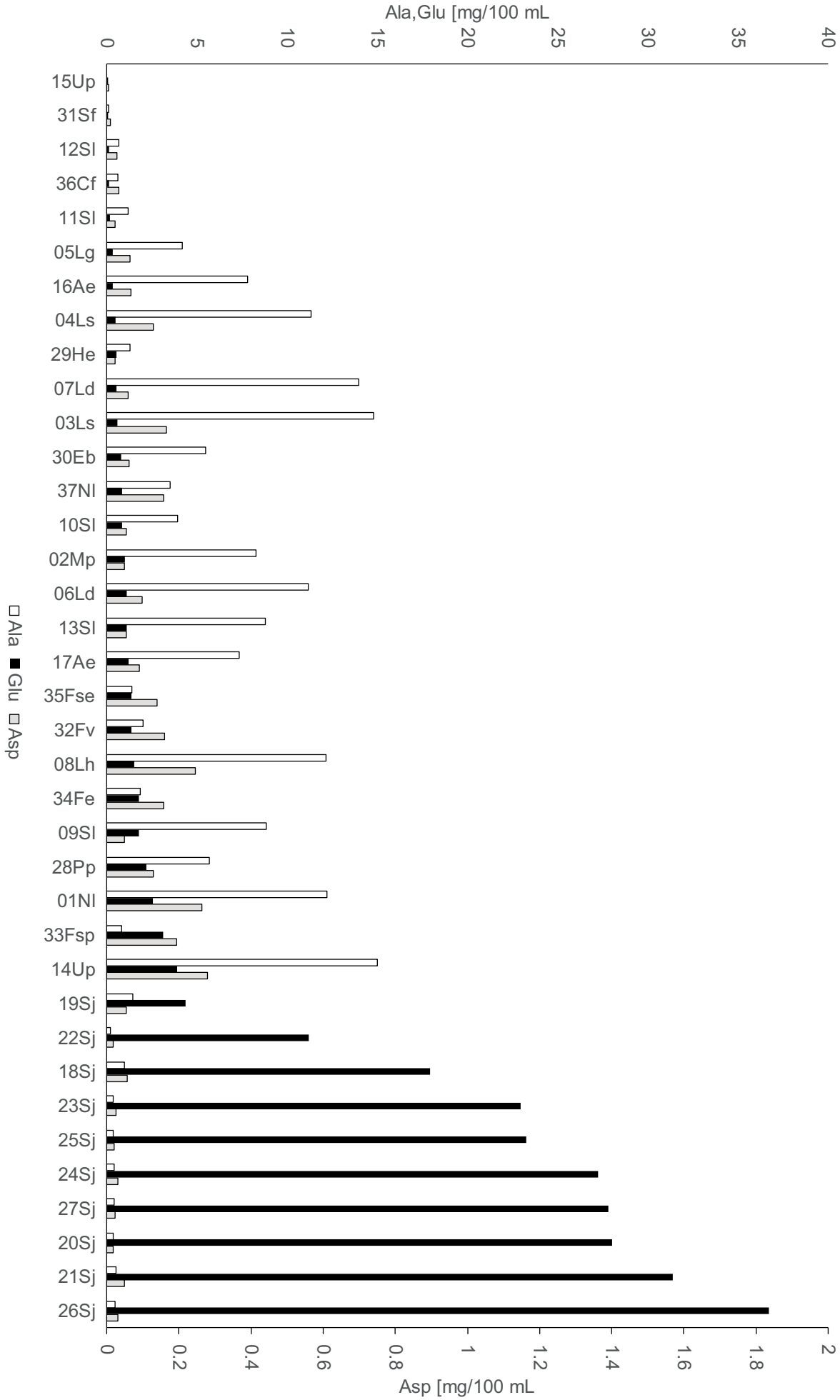

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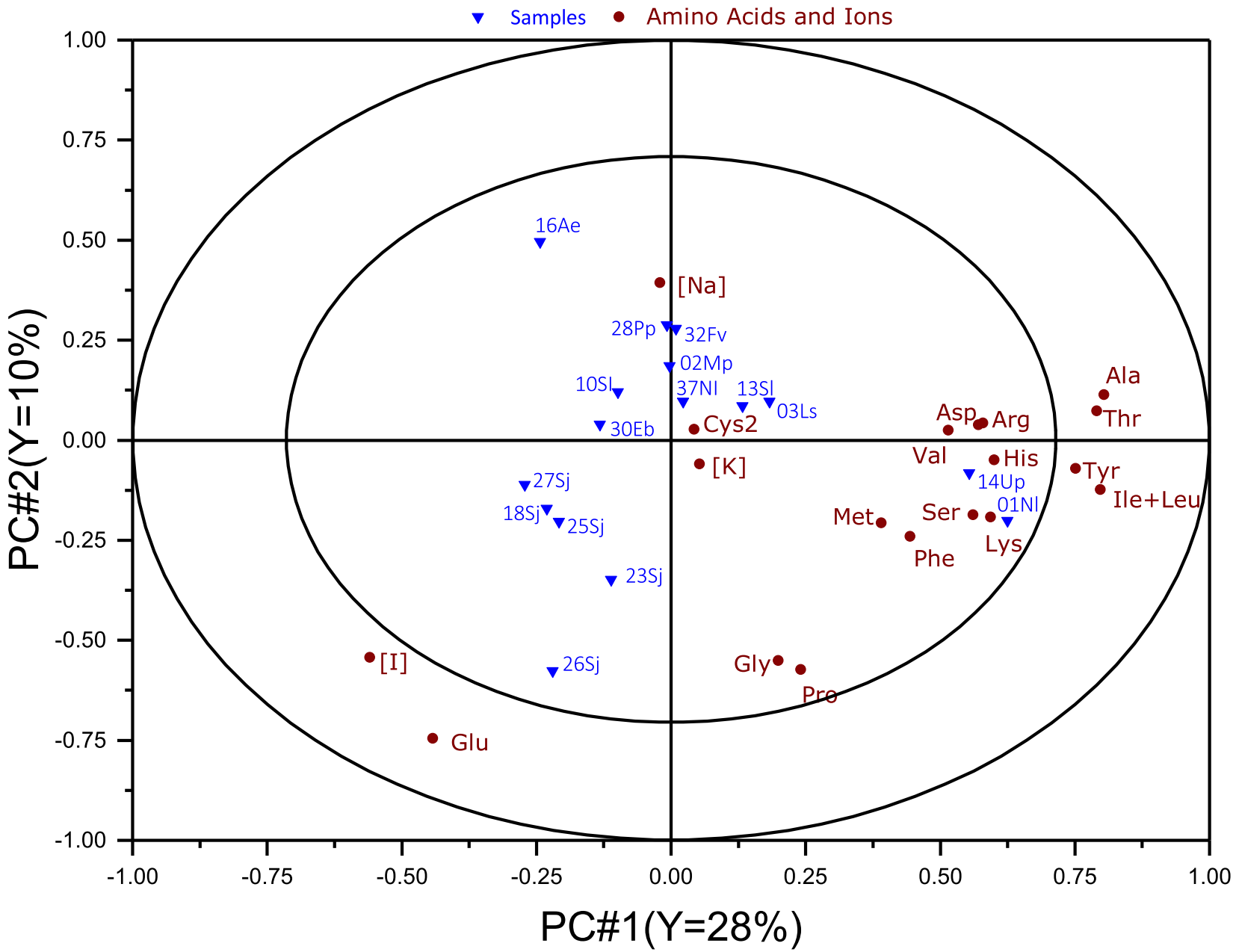

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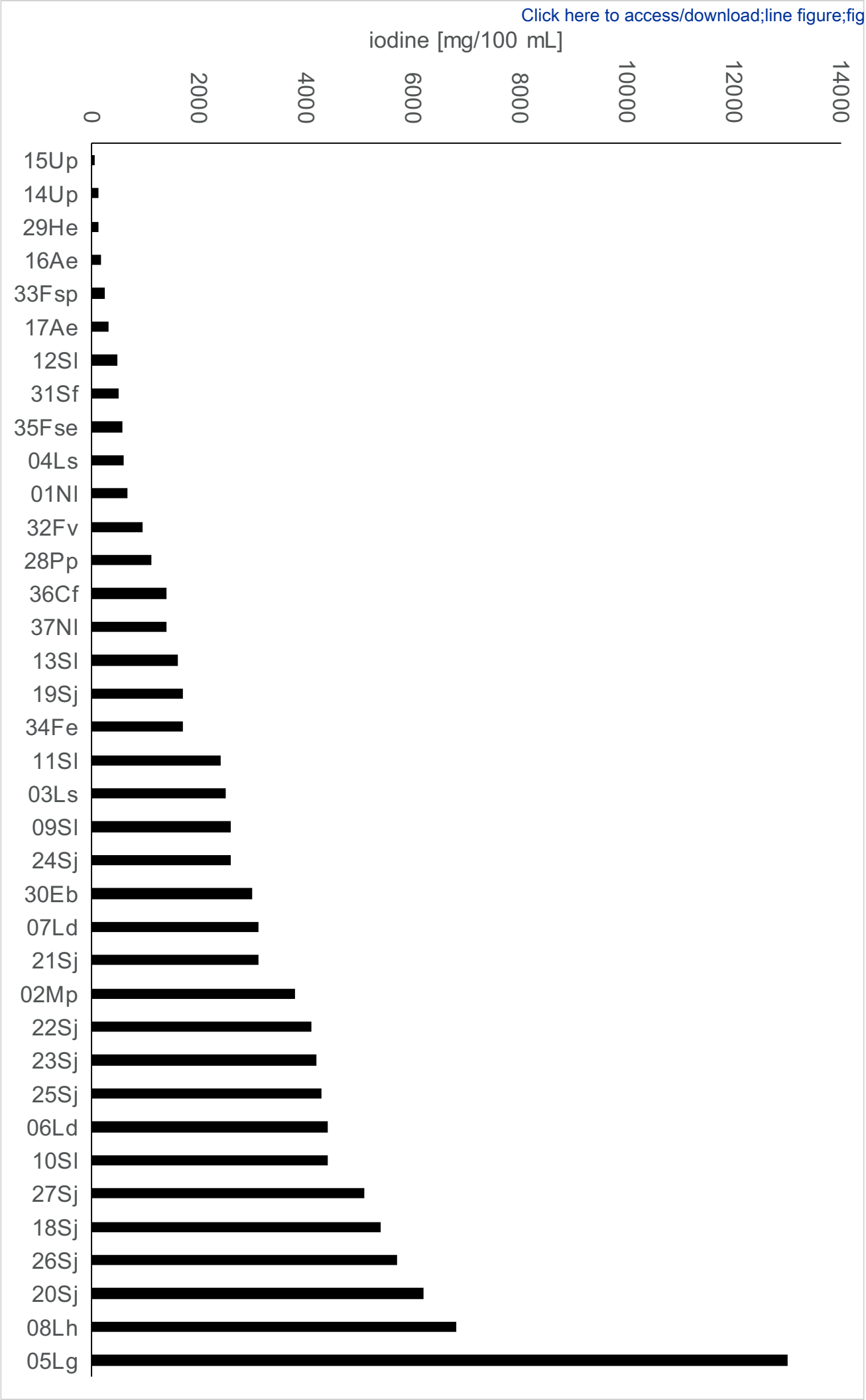

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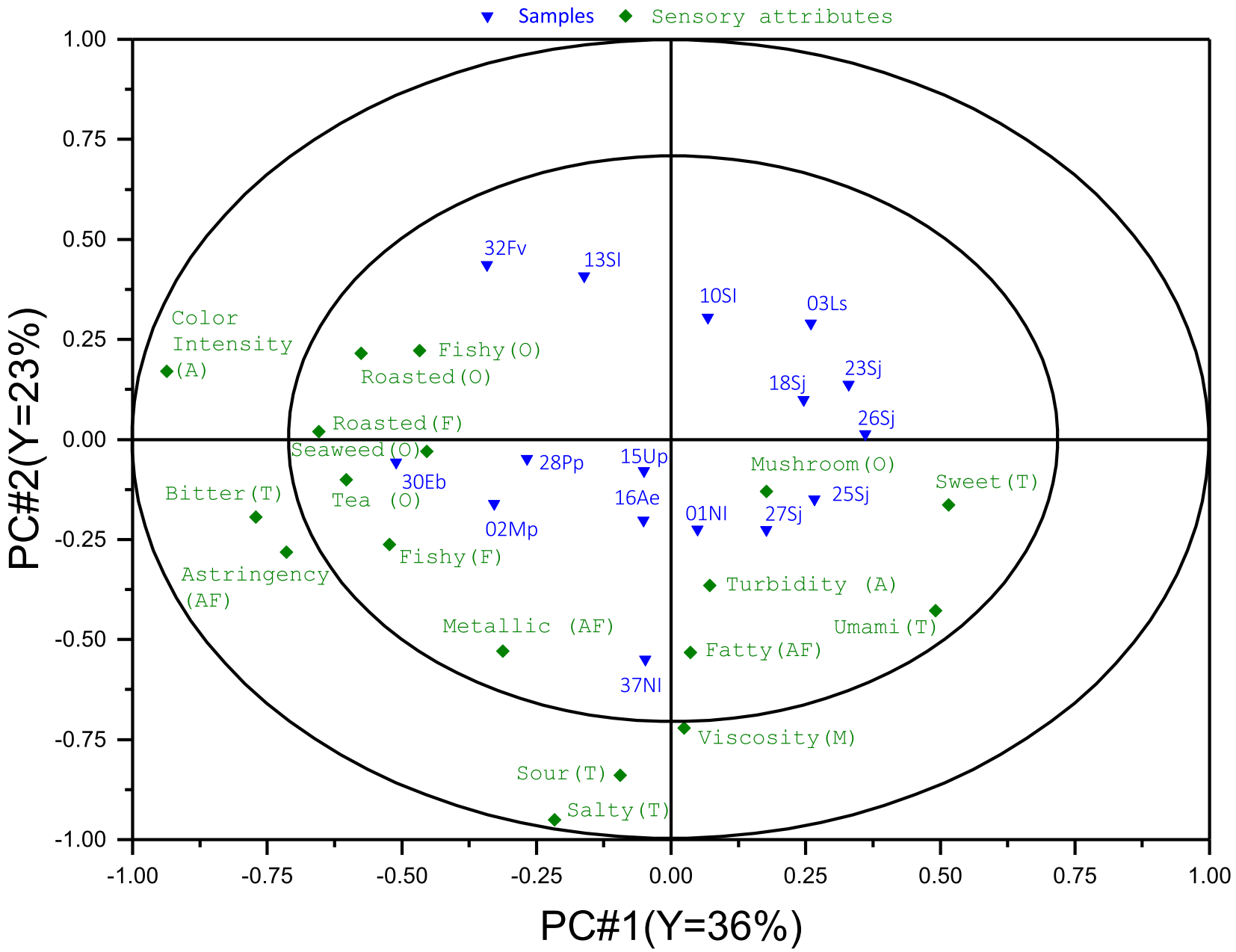

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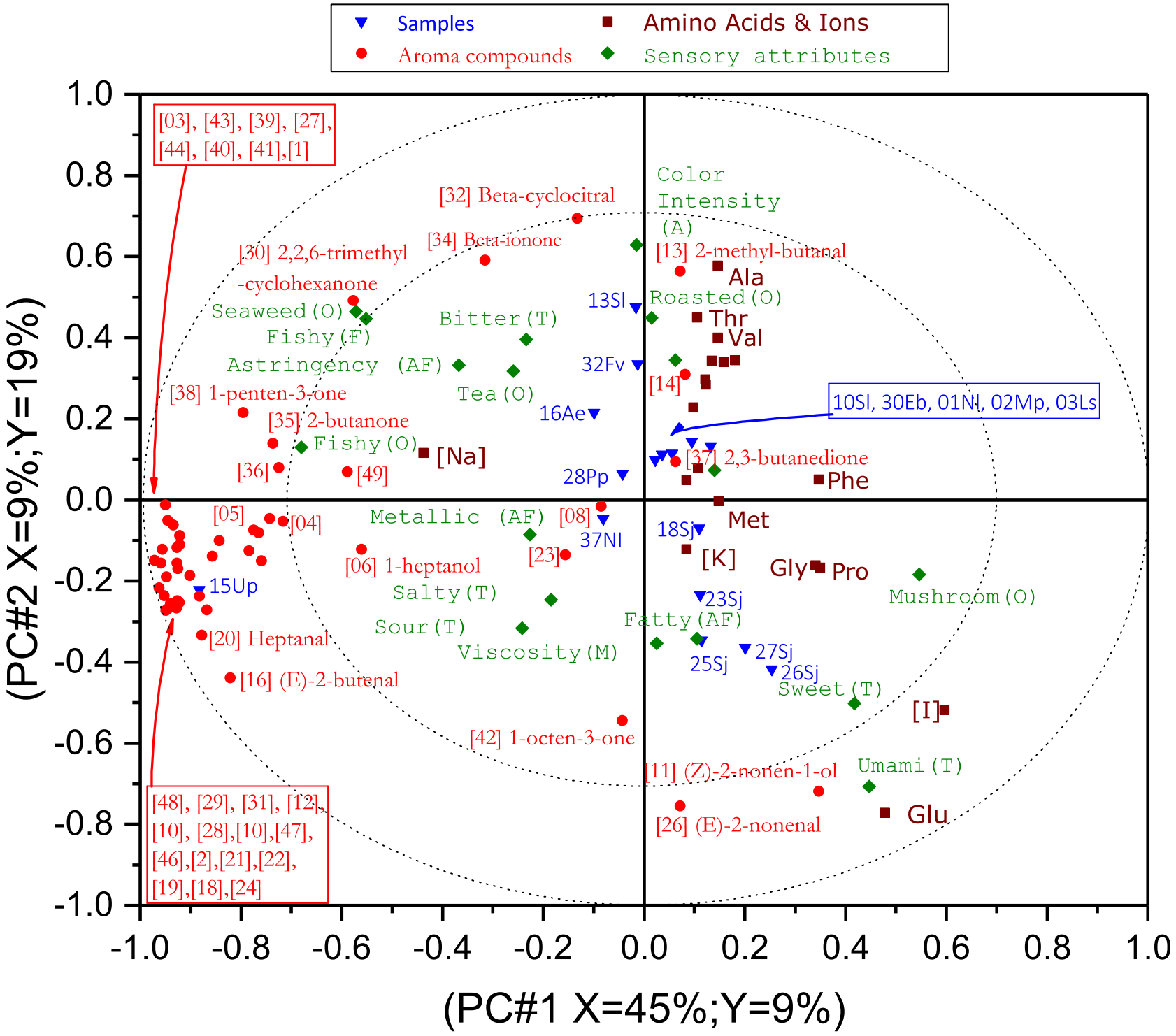

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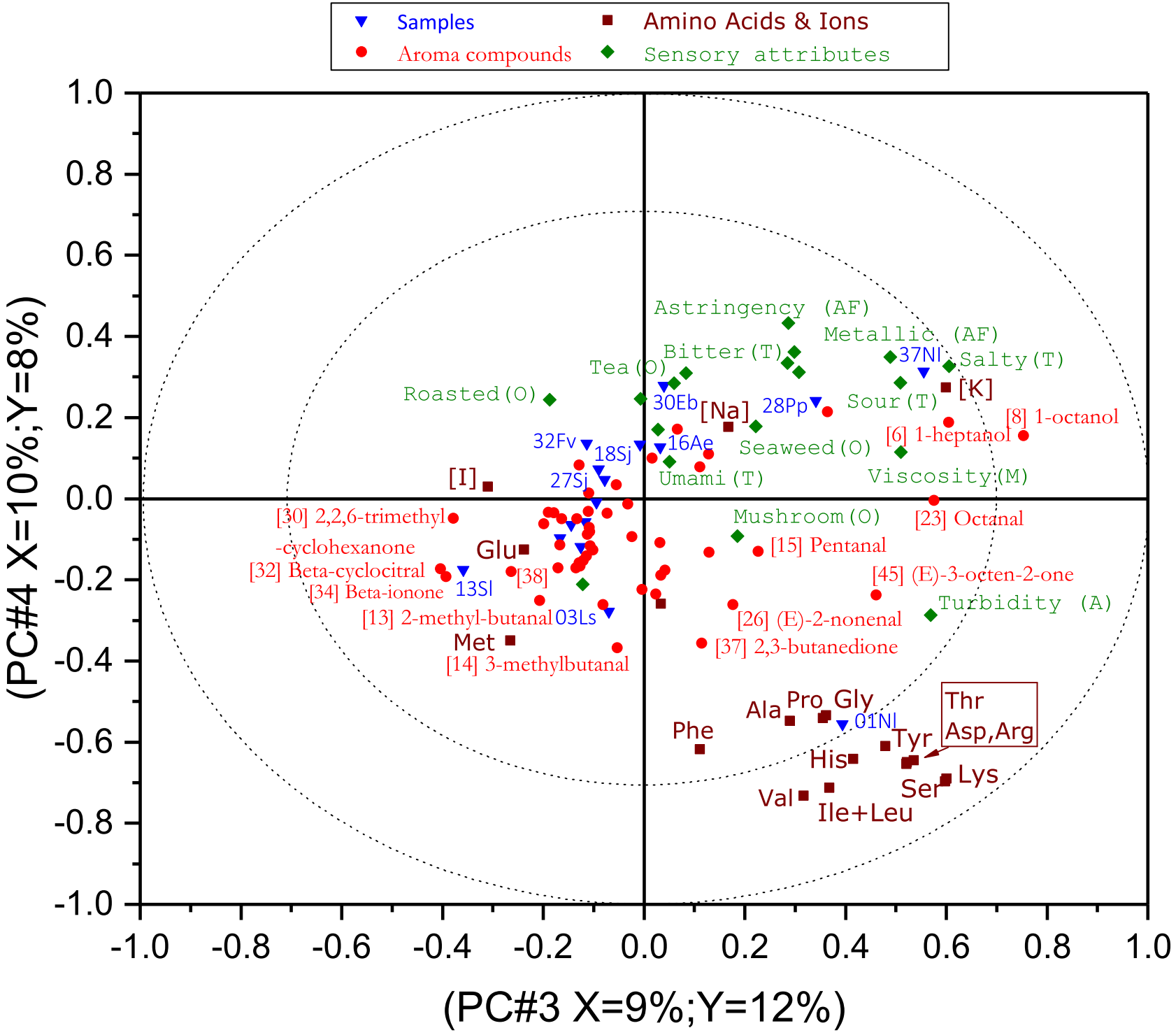

line figure

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